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NUTRITION REVIEWS

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PROBLEMS OF TROPICAL MALNUTRITION

Consideration of the nutritional aspects of tropical health should be disturbing to experimental, clinical, and public health nutritionists; to experimental nutritionists because most of the syndromes observed in the tropics are not reproducible as yet in the experimental animal—such diseases as malignant malnutrition, tropical ulcer, certain tropical anemias, primary carcinoma of the liver, have not been effectively studied in the laboratory; to clinical nutritionists because while the causal relationship of many tropical diseases to faulty diets is obvious, analysis of even the general lines of their etiology has often proved impossible, and responses to therapeutics, even when positive, have been baffling; to public health nutritionists because, generally speaking, the extensive development and sometimes even the appearance of many of the nutritional scourges observed in the tropics have accompanied colonization and "civilization" of tropical areas, particularly in Africa.

But first, why speak of specific nutritional problems in the tropics? Nutritional requirements of man in either hot and dry or hot and humid environments have been studied carefully, particularly during or immediately after World War II, and no striking deviations from "temperate" requirements (except for water) have been noted. True, increased losses in the sweat may increase requirements for certain minerals, but even this was shown to have been somewhat overdramatized and more characteristic of the acclimatization than of the acclimatized stage.

Increased requirements for thiamine, and perhaps for ascorbic acid, have been indicated, but again they do not justify separate consideration of tropical nutrition. The answer must be looked for in other fields.

An obvious and well-known factor is the concomitant prevalence of parasitic and infectious diseases in the tropics. Bacterial, protozoan, and metazoan parasites and, even more important, their commonest carriers do not regulate their body temperature. Their activity and reproductive rate are thus directly subjected to the environmental temperature and are many times higher in hot tropical environments than they are even in the "warm temperate" zone. This plus inadequate sanitation and hygiene largely but not entirely account for this prevalence. The importance of the pathogenic agents must not eliminate consideration of the "terrain," of the condition of the organisms affected. There, nutritional considerations are obviously paramount.

The other facts which warrant separate consideration of tropical malnutrition are neither physiologic nor clinical but agricultural, economic, and social. Colonizers have generally reduced the incidence of many bacterial and parasitic diseases by preventive inoculations, checks, quarantines, and sanitation campaigns such as area spraying. Thus, they have eliminated an unpleasant, but effective, form of population control.

At the same time, extensive deforestation and land use conducted on the basis of immediate profit, without due regard for the necessities of conservation, were often followed by erosion and drastic impoverishment of the soil. Sometimes rich lands were taken away from the natives by the colonizers. The indigenous populations were then either herded into poorer native reservations or reduced to the conditions of low paid laborers. Certain territories of southern Africa probably offer the most glaring examples of such concomitant wasting of both lands and peoples. The traditional diet of African populations, based on whole grains, vegetables and fruits, and relatively abundant animal

products was thus replaced by monotonous meals of starchy foods, often not indigenous to the country—corn, for example—and the increase in malnutrition has been explosive.

It would be outside the scope of this article to try to give even a brief enumeration of the pathologic conditions associated with nutrition and found in the tropics. The following examples illustrate four types of such diseases:

(1) Syndromes which are essentially of dietary origin (even though they may be complicated by parasitic and infectious conditions) such as beriberi, phrynoderma, certain ocular lesions, and Kwashiorkor.

(2) Conditions which are probably of nutritional origin—although there is still some disagreement on this point—such as tropical ulcer, many anemias, in particular "tropical pernicious anemia," and some forms of anemias of pregnancy, tropical sprue, and certain urolithiases.

(3) Conditions where dietary factors appear extremely important from consideration of incidence and etiology, but which are not generally thought of as nutritional diseases, such as primary carcinoma of the liver and certain fibroses of the pancreas.

(4) Diseases which are essentially nonnutritional in their primary causes but where nutritional factors are implicated either in the response to the pathogenic agent (*e.g.*, in malaria) or as a secondary effect of, for example, impaired intestinal absorption, as in many ankylostomiases and helminthiases.

Tropical syndromes which are, properly speaking, nutritional, vary largely in complexity from relatively simple beriberi and typical skin and eye lesions due to avitaminosis A to the extremely complex "malignant malnutrition." It should be realized that even in dealing with Asiatic beriberi, and even more so with pellagra—African forms in particular—there are generally deficiencies of more than one vitamin; the "burning feet" complication of beriberi is one such example.

Similarly, administration of vitamin A may clear one type of skin lesion, for example phrynoderma, and fail to clear concomitant facial skin lesions. But still, typically, and particularly in the acute form, these syndromes do respond dramatically to administration of the main missing elements, be they vitamin A, thiamine, niacin, or "good proteins."

The opposite is true of Kwashiorkor (*Nutrition Reviews* **4,** *52, 303 (1946)*; **6,** *270 (1948)*; **8,** *55, 161 (1950)*), an extremely widespread disease, first observed among the native populations of tropical and equitorial Africa, seen particularly in recently weaned infants, and characterized by a typical ("crazy pavement") dermatosis, graying of the hair even among infants, alopecia, skin pallor, chronic diarrhea, intensely fatty liver with or without cirrhosis, decreased plasma albumin and increased globulin, cystic fibrosis of the pancreas, often glomerular hyalinization of the kidneys and swelling of the parotid and other salivary glands, and edema. In Kwashiorkor one seems to be dealing with multiple deficiencies, with protein deficiency probably the constant factor, followed by a complicated series of pathologic changes, some of which are reversible in their early forms and many of which are irreversible and often fatal; hence the name "malignant malnutrition."

Kwashiorkor is always associated with the "plantation diet" of plantains, cassava, sweet potatoes, millet, corn, and ground nuts, with the "pap" gruel of guinea corn the most important staple in the diet of small children. If it is possible to treat it early by administration of a good diet, therapeutic response is good, although still far less striking than, for example, in pellagra. On the other hand, response to individual nutrients is inconclusive; the skin changes do not respond much to niacin therapy. While the short, intense coma rapidly followed by death resembles the picture of acute ariboflavinosis in the dog described by W. H. Sebrell and R. H. Onstott (*Pub. Health Reports* **53,** *83 (1938)*) and by H. R. Street and

G. R. Cowgill (*Am. J. Physiol.* **125,** *323 (1939)*), response to riboflavin is often minimal, particularly as regards fatty liver changes.

If the exclusively dietary origin of beriberi or Kwashiorkor is established beyond doubt, there still is some uncertainty as to the origin of diseases such as tropical ulcer, tropical sprue, and certain tropical anemias, though evidence is rapidly accumulating on their nutritional origin.

Tropical ulcer (M. V. R. Rao, *Proc. Fourth Internat. Congr. Trop. Med. Malaria, p. 1202, Washington, D. C. (1948)*; *Nutrition Reviews* **3,** *205 (1945)*) is characterized by rapid development of an exploding bleb on a previously sound skin surface, followed by a quickly destructive gangrenous process affecting muscles, tendons, nerves, vessels, and even the periosteum of bones. Its origin has previously been variously ascribed to spirochetes, fusiform bacilli, and diplococci; the disease now seems to have been conclusively ascribed to nutritional deficiencies by the studies of A. D. Charters (*Tr. Roy. Soc. Trop. Med. Hyg.* **37,** *205 (1943)*) who, during the war, compared the high incidence of this disease among East Africans with its extreme rarity among Somalis living in the same environment. Diet was the only variable, and a Somali diet proved curative to East African patients, vitamin A and calcium appearing to be the most important elements.

Similarly, evidence is rapidly mounting that tropical sprue (*Nutrition Reviews* **1,** *331 (1945)*; **4,** *11, 102, 147, 199 (1946)*; **8,** *62, 93 (1950)*; P. Manson-Bahr, *Proc. Fourth Internat. Congr. Trop. Med. Malaria, p. 1240, Washington, D. C. (1948)*), a disease characterized by a profound disturbance of the power of the intestines to absorb in a normal fashion the products of digestion with resulting steatorrhea and general diarrhea, macrocytic hyperchromic anemia, glossitis, a flat dextrose tolerance curve, and weight loss, is largely a nutritional disease although some observers still think it may have primarily an infectious origin because of its seasonal variations and frequent coexistence with dysentery. Yet it is generally associated with poor diets and shows hematologic response to folacin, vitamin B_{12}, and liver extract. The study of the role of microorganisms of the intestinal flora as well as the search for specific viruses impairing intestinal absorption may perhaps bring additional clues of its etiology.

Primary cancer of the liver (*Nutrition Reviews* **3,** *19 (1945)*) is a striking example of diseases which are often not thought of as nutritional but which, at least in some regions of the world, seem to be ascribable to diet. The incidence of primary cancer of the liver is relatively low among western peoples. For example, hepatic tumors accounted for only 0.17 per cent of the total number of patients admitted for neoplastic diseases to the Memorial Hospital, New York City, between 1917 and 1929.

On the other hand, the incidence of hepatic tumors is considerably higher in the southern half of Africa, Southeast Asia, China, and in the Asiatic Islands including Japan, the Philippines, Java, and Sumatra. For instance, C. Berman (*South African J. Med. Sci.* **5,** *92 (1940)*; **6,** *11, 145 (1941)*) has shown that among Bantus working in the South African mines, hepatic cancer accounts for 90 per cent of all cancers; among the general Bantu population the incidence is 37.4 per cent. The diet of these populations is poor, consisting essentially of corn ("mealie meal") and fermented milk with no meat and little vegetables. Various non-nutritional explanations have been proposed, none of them satisfactory: the high incidence of fibrosis of the liver among Bantus, alcoholism, liver parasites, and finally, genetic susceptibility. This last was disposed of by the careful comparison of the incidence in West Africa (characterized by a proportion of about 18 per cent of all cases of cancer due to hepatic cancer) to the distribution of incidence of cancer in the United States, where states like Alabama and South Carolina which contain a large proportion of

Negro descendants of West Africans present the lowest incidence of hepatic tumors, while almost "all-white" states like South Dakota and Indiana have the highest. The nutritional explanation must therefore be considered extremely seriously. So must it be for such conditions as gynecomastia, testicular atrophy and, generally speaking, "the feminization syndrome" which several keen African observers like J. N. P. Davies (*personal communication*) have commented upon.

A few brief comments on the relation of primarily non-nutritional tropical diseases to nutritional factors will be given. The effects of interference with proper intestinal absorption either directly or through impairment of bile production, with resulting danger of specific deficiencies as well as of general malnutrition, are too well known to warrant detailed consideration.

The relation of malnutrition to decreased resistance to infections may also be a factor, in particular, in the fulminating character of pulmonary tuberculosis among African natives. It may be more useful to recall demonstrations (*Nutrition Reviews* **1,** *362* (*1943*); **3,** *345* (*1945*)) of the importance of biotin for effective reaction to infection by certain types of *Plasmodium*, while on the contrary, pantothenic acid deficiency seems to decrease the severity of the infection, an illustration of the differential problems of nutrition involved in host-parasite relationships.

What pragmatic conclusions can be derived from consideration of these nutritional aspects of tropical medicine?

Generally speaking, it seems necessary to describe and map on the "geopathologic" scale, both diets, adequately described in terms of foodstuffs as well as in terms of nutrients, and prevalent syndromes. Striking correlations may thus become apparent.

Research workers should remember that the analytical approach, the careful observation of natural phenomena later put on experiment, is still a "royal avenue" to discovery. In particular, reproduction of tropical food habits, with "tropical" diets and the characteristic alternation of periods of caloric sufficiency and of undernourishment, may yield surprising results.

Clinicians should ponder the history of our knowledge of nutritional diseases where almost invariably the syndrome was first considered to be due to pathogenic agents, bacteria, viruses, or poisons, and wonder as to the number of such diseases which still have not been recognized as due to faulty nutrition.

Agricultural and economic planners of colonial nations as well as experts in "technical" assistance should meditate on the human consequences of the type of planning which does not first take into consideration the nutrition of the populations they want to help.

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DIET AND TOOTH DEVELOPMENT

Laboratory experiments and human surveys which indicated a strong relationship between the composition of the diet consumed during tooth development and the susceptibility to tooth decay have been described (*Nutrition Reviews* **6,** *196* (*1948*); **8,** *37* (*1950*)). When female rats, mice and hamsters were fed a diet composed of natural foodstuffs throughout pregnancy and lactation, the teeth of their offspring were quite resistant to decay; however, when female rats, mice and hamsters were fed, throughout pregnancy and lactation, a highly purified diet containing all nutrients known to be essential for well-being, their offspring were found to be highly susceptible

to the initiation and development of carious lesions. A similar effect of diet during tooth development was reported by various investigators in children of at least 11 European countries during the first and second World Wars. Varying degrees of reduction in the dental caries incidence of different populations and of different age groups were observed, but the maximum reductions did not occur until five or six years after the beginning of sugar and flour rationing. This time lag from the change of diet to the maximum reduction in tooth decay suggested that the effect had been mediated partly by the diet through the development of teeth which were less susceptible to decay rather than entirely by the alteration of the oral environment surrounding the teeth.

Since the above reviews have been published, additional data have been recorded by European investigators. One of the most detailed compilations of caries data from a war-torn country is that of T. H. J. Ekman (*Tandlaegebladet* **52,** *215 (1948)*). This Finnish investigator has recorded in detailed tables the disease rate in individual teeth of several age groups of public school children in Helsinki in 1938, 1940, 1942 and 1944. Thanks to Ekman's segregation of individual teeth and age groups, the data in this paper may well become known as the most important single tabulation of caries statistics recorded for any country during World War II. Not only the collection and segregation of the facts but the method in which the data are presented are commendable. Since the tabulated findings were published posthumously, they are unaccompanied by the author's concept of what they indicated. From inspection of his tables, one can only conclude that the younger children, and the older children who have recently erupted teeth, have benefited far more by the wartime regimen than those children whose teeth were formed prior to the war period. One of the clearest comparisons can be made in the age groups of children twelve to fourteen in 1938 and 1944. The permanent incisors and the first permanent molars of the twelve- to fourteen-year-old children in 1944 erupted in the period from 1936 to 1938; despite wartime restrictions in refined carbohydrates, the above teeth have very little less decay than the same teeth of children of this age group in the prewar year 1938. In contrast, the second permanent molars and the bicuspids of the twelve- to fourteen-year-old group in 1944 have erupted in the period just before the dental examination. Notwithstanding the fact that they have erupted into the same oral cavities and presumably into the same oral environments in which the first permanent molars and the permanent incisors have become carious, the second permanent molars and the bicuspids of the twelve- to fourteen-year-old group in 1944 have much less tooth decay than the same age group in 1938.

The effect of postwar dietaries on the dental caries incidence in European children has been the subject of much speculative curiosity on the part of dental investigators. Scattered data are beginning to become available in some cases for as late as 1948, or a total of three years after the end of hostilities. Just as great caution must be taken in connection with the interpretation of the postwar data as with that collected for the war period. If the facts are to mean anything insofar as understanding the causes of the variation in susceptibility to tooth decay, specific teeth and specific age groups must be discussed rather than the over-all change observed in a population in a community without respect to the individual teeth or to the age of the subjects. Insofar as facts are available, data for postwar years all show a definite trend toward maintenance of a much lower susceptibility to dental caries than was characteristic in prewar years. In groups of five-year-old children from London, England, M. Mellanby and H. Coumoulos (*Brit. Med. J.* **1,** *837 (1944)*; **2,** *565 (1946)*) and M. Mellanby and H. Mellanby (*Ibid.* **2,** *409 (1948)*) reported that the percentage with caries-free deciduous teeth was

zero in 1929, 14.9 in 1943, 24.2 in 1945 and 28.1 in 1947. Similar values are available for the deciduous teeth of five-year-old children in the communities of Blaker and Fet in Norway (R. F. Sognnaes, *Brit. Dent. J.* **87,** *291 (1949)*). The percentage of caries-free children were as follows: zero in 1939, 5 in 1944, 13 in 1945 and 26 in 1947. Further data from Norway are available for fourteen-year-olds (E. Alexander, *Norske tannlaegeforenings tidende* **58,** *367 (1948)*). The percentage of carious tooth surfaces was 16 per cent in 1939, 12 per cent in 1944 and only 5 per cent in 1948.

A slightly different approach which appears to contribute additional knowledge to the problem of the relation of wartime diets to the dental caries incidence of English children has been presented by R. Weaver (*Brit. Dent. J.* **88,** *231 (1950)*) in work dealing with the extent of reduction in tooth decay of five-year-old children in Cambridge. During the years 1925 to 1941, inclusive, an average of 19.6 per cent of the deciduous teeth of these children were carious. For the years 1942 and 1943, 14.1 per cent of the deciduous teeth were carious; for 1944, 1945 and 1946 the average percentage of carious deciduous teeth was 11.0. For 1947, 1948 and 1949, an average of 8.0 per cent of the deciduous teeth were decayed. The lowest incidence of tooth decay in the period from 1925 to 1949, inclusive, was observed in 1949 when only 6.7 per cent of the deciduous teeth of five-year-old children were carious. This decrease was observed four years after the official end of the war even though there had been a national trend toward a return to peacetime dietaries. In view of this, and a prolonged remarkable decrease in caries susceptibility, this investigator was interested in determining whether similar reductions had occurred in regions where there was a low caries incidence in the prewar period because of the presence of naturally occurring fluorides in the drinking water supply.

Since Weaver had previously made a survey of the influence of fluorides on tooth decay in five- and twelve-year-old children in two British communities in 1943, (*Brit. Dent. J.* **76,** *29 (1944)*), he chose to re-examine the teeth of the same age groups again in 1949. There were 500 children in each age group of each survey in each community. The community of North Shields had no fluoride in the drinking water, while the communal water in South Shields contained 1.4 parts per million (p.p.m.) of fluorine as fluorides. In 1943, the five-year-old children in North Shields had an average of 6.6 decayed, missing or filled (DMF) deciduous teeth, while the twelve-year-old children had an average of 4.3 DMF permanent teeth. In contrast, in South Shields the incidence of tooth decay for five-year-olds was an average of 3.9 DMF deciduous teeth and for twelve-year-olds was an average of 2.4 DMF permanent teeth. In 1949, in North Shields the average number of diseased deciduous teeth among five-year-old children was 4.4 instead of 6.6, while the average number of DMF permanent teeth in twelve-year-olds was 2.4 in contrast to 4.3. In South Shields, where there were fluorides in the drinking water, there was only a slight and probably insignificant reduction in the average number of diseased deciduous teeth among five-year-old children during the years between 1943 and 1949 (3.9 versus 3.5 DMF, respectively). Among twelve-year-old children in South Shields, wartime diets did result in a reduction in tooth decay from 2.4 to 1.3 DMF permanent teeth. This reduction was percentage-wise quite similar to the reduction resulting in North Shields from consumption of wartime diets in the absence of fluorides in the drinking water. A further interesting comparison made in this survey was that 25.8 and 50.6 per cent of the twelve-year-old children in South Shields in 1943 and 1949, respectively, had no DMF permanent teeth, whereas only 4.8 and 26.4 per cent of the same age group in North Shields in 1943 and 1949, respectively, had no diseased or missing permanent teeth. In other words, the effect of wartime diets and of

fluorides appeared to be additive insofar as permanent teeth were concerned.

In the 1949 survey, an additional community, West Hartlepool, which had 2.0 p.p.m. of fluoride in the drinking water supply, was surveyed for dental disease manifestations. The 500 children in the five-year-old group had an average of 1.8 DMF deciduous teeth and 53.6 per cent of the children were free of tooth decay. The 500 children in the twelve-year-old age group had an average of 1.0 DMF teeth and 59.8 per cent of the group had no caries.

A further comment by Weaver strengthens the inference that wartime diets did not produce their beneficial effects insofar as the formation of caries-resistant teeth was concerned through a fluoride relationship. He pointed out that in areas where fluorides normally occurred in the drinking water the percentage reduction in caries was greatest in the incisor teeth and least in the molar teeth, whereas wartime diets appeared to be approximately equally effective in increasing the caries resistance of all teeth developed during systemic exposure to these diets, irrespective of their normal position in the oral cavity.

The exact mechanisms by which wartime dietary regimens influenced the caries resistance of teeth are unknown today. The evidence strongly points toward beneficial aspects of well-balanced natural diets which can not be expressed in terms of presently recognized standards for known nutritional essentials.

BIOLOGIC SYNTHESIS OF METHYL GROUPS

As the result of a variety of investigations carried out during the past decade the concept has evolved that the methyl group is a factor of considerable importance in mammalian nutrition. Although the methyl group is of relatively simple organic structure and configuration, dietary sources are required (*Nutrition Reviews* **2,** *358* (*1944*); **4,** *163, 306* (*1946*); **5,** *131* (*1947*)). Most significant as dietary constituents supplying methyl groups are choline and methionine. These substances provide labile methyl groups which are readily available for processes of transmethylation. It has been found that there is free interchange of methyl groups between choline and methionine (E. B. Keller, J. R. Rachele, and V. du Vigneaud, *J. Biol. Chem.* **177,** *733* (*1949*)). Among the substances which may replace these methyl donors in the diet are betaine and dimethylthetin. The physiologic importance of methyl donors resides in their functions as lipotropic agents, in prevention of hemorrhagic lesions of the kidney and in promotion of body growth.

Until relatively recently it was presumed that the dietary requirement for methyl donors resulted from inability of the body to synthesize compounds which could contribute labile methyl groups for utilization in transmethylation. Within the past year reports have appeared which provide preliminary but apparently conclusive evidence of the biosynthesis of methyl groups of choline from methanol and formate as precursors (*Nutrition Reviews* **8,** *269* (*1950*)). Confirmation of these observations is found in the results of experiments reported by H. R. V. Arnstein of the National Institute for Medical Research in London (*Biochem. J.* **48,** *27* (*1951*)). The experimental background lay in the demonstration that the labeled carbon of the methyl group of choline when fed to rats appeared in the β-carbon atom of the amino acid, serine, and that serine was synthesized in the animal body from formate and glycine (W. Sakami, *J. Biol. Chem.* **176,** *995* (*1948*); **178,** *519* (*1949*)). An attempt was therefore made to see whether these reactions moved in the reverse direction with the carbon atoms of relatively simple compounds

serving as precursors for the methyl carbon of choline.

White rats of the Wistar strain were used in these experiments. Animals were fed a stock diet adequate in content of choline and methionine and therefore in labile methyl groups. To the diet was added one or the other of a group of relatively simple compounds labeled with C^{14} which were being tested as carbon donors in the biologic synthesis of choline. The precursors administered were D,L-, L-, and D-serine carrying the tag in the β-carbon, glycine labeled in the α-position or in the carboxyl carbon, and sodium formate and methanol containing C^{14}. The labeled compound was fed during the period of one night and the animals were killed after a period of from one to five days. Livers were removed, following which the carcasses of the animals were minced, and extracted with ethanol. Choline was isolated as the chloroplatinate and then degraded to trimethylamine. Radioactivity of the choline and trimethylamine isolated was determined. Evidence was presented to substantiate the view that the carbon atoms of trimethylamine, thus derived, originated in the methyl groups of choline.

By these means it was demonstrated that the methyl groups of choline may be synthesized within the animal body. Sodium formate, methanol, glycine labeled in the α-position, and L-serine with C^{14} in the β-position proved to be effective precursors. In contrast the carbon tag included in the carboxyl carbon of glycine or in the β-carbon of D-serine did not later appear in the methyl group of choline. Degradation of the choline showed that the carbon of methanol was more effective as a precursor of the choline methyl groups than were carbon atoms in the other compounds also demonstrated to function as precursors. In addition, it was found that when glycine labeled with N^{15} was fed, the isotopic nitrogen was incorporated into choline at approximately the same rate as C^{14} administered in glycine tagged in the α-position.

These observations are of significance because they again demonstrate biosynthesis of labile methyl groups from relatively simple compounds. It should again be pointed out that the experiments were carried out on animals consuming diets which were in no way inadequate in content of labile methyl groups. It must be assumed that *in vivo* synthesis does not take place at a rate which is sufficient to permit optimal growth or effectively inhibit fat deposition. It is perhaps for this reason that a dietary source of labile methyl groups is required.

There are other observations of interest in the report of Arnstein (*loc. cit.*). For instance, the variance in metabolic pathways achieved by stereoisomers is demonstrated in that the β-carbon atom of L-serine is shown to find its way into choline, whereas that of D-serine did not function as a precursor of choline.

Evidence is deduced to show that ethanolamine is derived from serine with loss of the carboxyl group. In these experiments it was found that L-serine with the tag in the β-carbon was converted to choline with about the same efficiency as glycine carrying N^{15} as a tag. The same was true for glycine labeled in the α-carbon. However, the carboxyl carbon of glycine was not converted to choline. Since glycine is known to serve as a precursor of choline, and since glycine and serine are in dynamic equilibrium and the carboxyl carbon of glycine appears only in the carboxyl group of serine, the synthesis of the ethanolamine moiety of choline from glycine must be by way of serine with loss of the carboxyl group.

The author considers two possible one-carbon compounds as precursors of the methyl group. The first mentioned is formate, since formate and formaldehyde have been demonstrated to be degradation products of the β-carbon of L-serine as well as of the α-carbon of glycine (P. Siekevitz and D. M. Greenberg, *Fed. Proc.* **9,** *227 (1950)*). However, the carbon of methanol was converted to choline more efficiently than that

of formate. Therefore, another type compound is suggested as precursor. Specifically it is considered likely that a phosphorylated one-carbon compound derived from the β-carbon atom of serine during its conversion to glycine might exist as precursor. Such a compound has not been isolated and identified. However, the demonstration of the biosynthesis of labile methyl groups has been quite conclusively achieved. The exploration of the intermediary pathways involved and the isolation of precursors remain for future experimentation.

BLOOD PRESSURE AND BODY COMPOSITION ON THE RICE-FRUIT DIET

For a half-dozen years the use of the rice-fruit diet has been in the center of attention as the latest of a series of attempts to treat hypertension by dietary means (*Nutrition Reviews* **8,** *304* (*1950*); C. B. Chapman and T. B. Gibbons, *Medicine* **29,** *29* (*1950*)). Observations on the effects of the rice-fruit dietary regimen were summarized by its originator, W. Kempner (*Am. J. Med.* **4,** *545* (*1948*)). The author considered not only the changes in blood pressure and several other criteria of the status of the cardiovascular system (electrocardiograms, retinal vessels, heart size) but also some of the chemical changes produced by the rice diet. However, these data are not sufficient for evaluation of the physiologic meaning of some of the changes accompanying treatment of hypertension with the rice diet, notably the decrement in body weight. Kempner considered the possibility that the food intake may not cover the caloric requirements but stressed that the most frequent cause of the weight decrement is the loss of visible or invisible edema.

The effects of the rice-fruit diet on the composition of the body were studied in detail by C. B. Chapman, T. Gibbons, and A. Henschel (*New Engl. J. Med.* **243,** *899* (*1950*)). The subjects were 8 men with definite, moderately severe essential hypertension. Their universal complaint was headache. Some noted dizziness and dyspnea on exertion but none had frank congestive failure or renal insufficiency. The patients were housed in a special hospital ward and strict dietary control was maintained.

The experiments included a prolonged control period (twenty-five to fifty-four days), a period of rice-fruit diet (twenty-two to one hundred and two days), a period of rice-fruit diet plus added sodium-free protein (fourteen to forty-two days), and, in 3 subjects, a period of rice-fruit diet plus salt (twenty-two days). The control-period diet provided daily about 3000 calories, 100 g. of protein, 150 g. of fat and 8 to 12 g. of sodium chloride. The rice-fruit diet contained slightly over 2000 calories, 22 g. of vegetable protein, 3.3 g. of vegetable fat and about 20 mg. of sodium per day, and was supplemented with vitamins. Dialyzed milk powder was used as the source of sodium-free protein (40 g. daily). The supplementary sodium chloride capsules provided 10 g. of salt daily.

On the average, the blood pressure dedecreased from the control value of 189/126 mm. Hg. to 159/105 mm. Hg. during the final week of the rice-fruit diet period. Headache became less intense or absent in 6 out of the 8 patients. Improvement in the other symptoms was less impressive. Electrocardiographically, no pronounced deviations were present during the control period and none were observed during the rice-fruit diet test period. The diet resulted in no improvement in the eyeground changes. In 2 patients who had a moderate cardiac enlargement at the start of the diet there was a definite decrease in the heart size after five weeks of rice-fruit diet treatment. It was noted but not documented by figures that the dietary regimen reduced the reactivity of the organ-

ism as measured by the cold-pressor and the sodium amytal tests.

All 8 subjects showed a loss of body weight. In 6 subjects on whom complete data were available, the body weight decreased from 78.5 to 69.8 kg. What type of body compartments contributed to this loss? The bone density, studied by radiologic methods, had not changed after twenty-four weeks of semistarvation, with a 24 per cent loss of body weight (A. Keys *et al.*, "The Biology of Human Starvation," *Univ. of Minnesota Press, Minneapolis (1950)*) and there is no reason to postulate any demineralization of bones in the rice-fruit diet experiment. In contrast to the semistarvation experiment, in which there was a small absolute (16.4 kg. in control, 17.9 kg. in starvation) and a large relative (23.6 and 34.0 per cent of body weight) increase in the extracellular fluid as measured by the thiocyanate space, the value decreased from 18.5 to 15.8 kg. on the rice-fruit diet. This difference is attributable to the salt intake, which was very low in the rice-fruit diet and high in the starvation experiment. The specific gravity of the body increased from 1.054 to 1.062 and the estimated fat content of the body (E. N. Rathbun and N. Pace, *J. Biol. Chem.* **158,** *667 (1945)*) decreased from 17.5 to 12.9 kg.

In addition to the loss of these "inactive" tissues, there was some loss of the "active" body tissues, presumably mostly the muscle (from 39.3 to 38.1 kg.). This value was obtained by subtracting the combined weight of fat, extracellular fluid, and bones from the total body weight. This would indicate that at least some of the men were in a negative nitrogen balance. Biochemically, this has been observed by W. B. Schwartz and J. K. Merlis (*J. Clin. Invest.* **27,** *406 (1948)*). In the experiment carried out by Chapman *et al.* (*loc. cit.*) the addition of 40 g. of salt-free protein reduced or stopped the loss of body weight, without affecting the blood pressure. On the other hand, addition of 10 g. of sodium chloride increased the blood pressure from 129/87 mm. Hg. to 165/103 mm. Hg. within three weeks, was associated with an increase in the extracellular fluid volume, and caused a prompt return of the symptoms.

One of the marked biochemical effects of the rice-fruit diet was a lowering of the serum cholesterol level from the average control value of 233 mg. per cent to 158 mg. per cent after thirty-three days on the diet. The decrease in the serum cholesterol level from a normal level is not likely to play a specific role in the observed lowering of the blood pressure. In the reduction of the blood pressure the salt restriction is certainly important but the specific mechanism remains uncertain and is probably complex. It was observed, both under laboratory and field conditions (J. Brozek, C. B. Chapman, and A. Keys, *J. Am. Med. Assn.* **137,** *1569 (1948)*), that semistarvation alone with no restriction in salt intake reduces blood pressure in both normotensive and hypertensive individuals.

NUTRITIVE VALUE OF IRRIGATED VERSUS NONIRRIGATED VEGETABLES

In times past, vegetables grown in the eastern part of the United States, close to their market, have depended upon natural rainfall for their development and have thus reflected the inequalities of different and constantly changing climatic conditions. Within the past four or five years irrigation has been practiced in a limited way in this section of the country, to improve both yield and the probability of a profitable crop. In many cases the quality of the food crop has also been improved by irrigation, as judged by appearance, succulence, tenderness, and other factors contributing to consumer acceptance. In the western states where irrigation has long been accepted as a necessary

practice during most of the growing season, the question of the effect of irrigation on quality is often a purely academic one, but this new trend in eastern states brings the question to the forefront. The soils in various parts of the country, as well as temperature and moisture conditions, vary over wide ranges. It might be assumed that an abundant water supply under eastern conditions might improve the quality of vegetables to the point where they might compete more equitably and more consistently in eastern markets with western vegetables shipped from afar. At any rate, the practice of irrigation will tend to minimize the effect of one more important variable in the environment of plants grown in eastern states.

One of the first crops investigated with regard to this question was snap beans. B. E. Janes (*Proc. Am. Soc. Hort. Sci.* **51,** *457 (1948)*) analyzed two varieties of snap beans grown at Gainesville, Florida, under different moisture conditions and found the expected difference in percentage dry weight favoring those grown without irrigation. Differences in carbohydrate, organic nitrogen, sugars and mineral content were small when calculated on a dry weight basis.

Since it is characteristic of fruits to be somewhat uniform in composition, regardless of growth conditions, Janes considered it important to investigate a crop that consisted primarily of leaf tissue, which might be expected to show more variation under differing growing conditions. He studied the composition of cabbage as affected by soil moisture (influenced by irrigation), nitrogen level of nutrition, and season. He also compared quality (consumer acceptance) with the chemical nature of the crop (*Plant Physiol.* **25,** *441 (1950)*).

Cabbage, variety Glory of Enkhuizen, was grown at Gainesville, Florida, with 4 levels of water supply (including no irrigation), 2 levels of nitrogen fertilization, and in 2 different seasons. Irrigation was applied by overhead sprinklers. One planting was started in warm weather (October) and harvested in cool weather (January), the plants maturing over a long period of time. The other was planted in cool weather (February) and harvested in warm weather (April), resulting in uniform maturity. Irrigation was applied frequently (every second day), moderately (every fourth day), and occasionally (every eighth day). During the first growing season, well-spaced rains and cool weather tended to minimize effects of irrigation, as shown by a constant fresh weight per head and percentage dry weight. An extended dry period late in the second season caused the irrigated heads to weigh almost twice as much as the nonirrigated and they also had a higher moisture content, as well as being of the highest quality.

On a fresh weight basis, the inorganic ions as well as the reducing sugars, acid hydrolyzable carbohydrates and ascorbic acid contents were remarkably constant for the entire first crop. With the second crop, ascorbic acid and acid hydrolyzable carbohydrates were 30 per cent higher in the occasional and nonirrigated, reducing sugars were identical, and differences in inorganic constituents were somewhat less, with some variation. On a dry weight basis, many of these differences disappear or become less significant. The net effect on total carbohydrates is one of reduction as irrigation is reduced. The per cent of nitrogen increased with decrease of irrigation.

Side dressings of sodium nitrate increased only the sodium content of the first crop. In the second crop, increase of nitrogen added to the soil was associated with an increase in the nitrogen content and decrease in carbohydrate content of the plant.

These studies indicate that for cabbage the physical characteristics, such as succulence, are more important than chemical composition in determining quality from the consumer's viewpoint. Significant differences in nutritional quality do not appear to arise from variance in irrigation procedure. The desirability of increased yield and improved

quality, as derived from irrigation, becomes a matter of economics.

Related studies in Oregon (conducted in greenhouses) by A. S. Hunter, W. C. Kelley, and G. F. Somers (*Agronomy J.* **42,** *96 (1950)*) were conducted on turnip greens grown at 2 fertility levels under 3 moisture conditions. Irrigation was applied when the moisture was exhausted at the 6 inch depth to tensions of 0.25, 0.85, and 15 atmospheres. Dry conditions (near the wilting point) at harvest produced small plants. On the fresh basis, the content of ascorbic acid increased under drier conditions, but not as much as the per cent dry weight. Possibly the maximum accumulation of ascorbic acid was prevented by moisture stress. Tough wilted leaves resulted from dryness. It is doubtful that within the range over which tender succulent leaves can be grown, variation of soil moisture has practical effects on the content of either ascorbic acid or carotene. These variations may, however, account for some differences observed in different locations and in different years. These data show the desirability of reporting results of analyses on the dry weight basis when soil moisture may be variable.

Plants under high soil moisture tension (dry) had a lower yield, higher percentage dry weight, and higher ascorbic acid and carotene contents on the fresh weight basis but less ascorbic acid on the dry weight basis than did plants grown under lower moisture tensions.

We may expect more attention to be given to irrigation in its relation to crop quality in eastern and southern states as economic pressure induces the necessity for increased yields.

ROLE OF STEROLS IN COUNTERACTING GROWTH DEPRESSION FROM HIGH-ALFALFA FEEDS

Poultry nutritionists have observed for a number of years that chicks manifest a distinct dislike of alfalfa meal. This was originally demonstrated by giving chicks a free choice of feed stuffs (W. R. Graham, *Poultry Sci.* **11,** *365 (1932)*).

In 1948 C. I. Draper (*Poultry Sci.* **27,** *659 (1948)*) and W. T. Cooney, J. S. Butts, and L. E. Bacon (*Ibid.* **27,** *828 (1948)*) reported that when either sun-cured or dehydrated alfalfa was present in chick diets at levels of 10 to 15 per cent, a significant decline in weight gain resulted. These investigators concluded that some unidentified factor present in alfalfa was responsible for the growth depression.

W. M. Insko and T. G. Culton (*Poultry Sci.* **28,** *769 (1949)*) observed that different samples of alfalfa meal varied greatly in their effects on chick growth. B. W. Heywang (*Ibid.* **29,** *804 (1950)*) likewise noted that meals varied considerably in their growth-inhibiting properties, and observed further that egg production was adversely affected as the alfalfa meal was increased from 10 per cent of the diet to 15, 20, or 25 per cent.

Although B. Adler (*Poultry Sci.* **27,** *651 (1948)*) had reported that 8-weeks-old turkeys could be fed diets containing 35 to 40 per cent of alfalfa meal without adverse effects, H. L. German and J. R. Couch (*Ibid.* **29,** *841 (1950)*) reported that growth inhibition in turkey poults was even more pronounced than that in chicks when they were fed a diet containing as low as 10 per cent of alfalfa meal.

S. Lepkovsky, W. Shaeleff, D. Peterson, and R. Perry (*Poultry Sci.* **29,** *208 (1950)*) considered the possibility that the Maillard reaction (*Nutrition Reviews* **8,** *193 (1950)*), sometimes referred to as the "non-enzymatic browning" of foods or feeds, might be involved in the alfalfa curing process. These workers concluded that the growth inhibitor was probably organic in nature, that it was stable to heat treatment, and that it could be

extracted with hot water. Further studies (Peterson, *J. Biol. Chem.* **183,** *647 (1950)*) indicated that the harmful agent was a saponin or saponin-like material. Peterson also observed that dietary cholesterol supplements counteracted to a large extent the growth-depressing effect of alfalfa meal or the inhibitory extracts of the meal.

More detailed studies of the relation of sterols to growth inhibition by alfalfa meal were recently reported by D. W. Peterson (*J. Nutrition* **42,** *597 (1950)*). Chicks, 7 to 14 days old, previously maintained on a normal stock diet, were fed the experimental diets for three weeks. The basal diet consisted of the following in per cent: ground barley, 30; ground corn, 22; ground wheat, 10; wheat bran, 10; soybean oil meal, 10; fish meal, 7.5; dried whey, 2.5; dried skim milk, 2.5; liver meal, 2; limestone, 1.5; bonemeal, 1.2; sodium chloride, 0.5; manganese sulphate, 0.025; and fortified fish oil (2250 I. U. of vitamin A and 400 I. U. of vitamin D per gram), 0.25. Riboflavin was added to the extent of 2 mg. per kilogram. Dietary substitutes were made at the expense of the barley, and the alfalfa meal samples were fed at a level of 20 per cent of the diet.

The first experiment was designed to determine if the effects of cholesterol in counteracting the growth depression by alfalfa meal could be enhanced by increasing the fat content of the diet. It was found that a 1.0 per cent dietary cholesterol supplement largely overcame the growth depression produced by diets containing 20 per cent alfalfa meal. Cottonseed oil alone was ineffective but the inclusion in the diet of both cottonseed oil (4 per cent of the diet) and cholesterol (0.5 to 1.5 per cent of the diet) completely prevented the harmful effects of alfalfa meal.

Average total blood plasma cholesterol values were increased whenever cholesterol supplements were included in the diet. They rose from an average of 58 mg. per cent in the plasma of chicks fed the basal diet containing 20 per cent alfalfa meal, to an average of 594 mg. per cent when the same diet contained 1.5 per cent of cholesterol and 4 per cent of cottonseed oil. In all cases, chicks that received both cholesterol and cottonseed oil had considerably higher blood cholesterol levels than those receiving cholesterol alone.

The second experiment was designed to establish whether or not increased blood cholesterol levels induced by estrogenic therapy would alleviate the harmful effects of alfalfa meal. Chicks that received subcutaneous implants of 15 mg. of diethylstilbesterol exhibited the same growth depression on the alfalfa-containing diets as did the controls, even though the blood cholesterol levels in the estrogen-treated chicks were markedly increased.

In the third experiment the effectiveness of plant sterols was determined. Soy sterols, consisting mainly of sitosterol and stigmasterol, were fed in comparison with cholesterol and were found to be equally as effective as cholesterol in restoring normal growth in chicks receiving the alfalfa-containing diets. Similar results were obtained with sterol fractions of tall oil.

Analysis of the blood demonstrated that blood sterols were not altered by the addition of plant sterols to the diet, and thus it was established that blood sterol concentration was not a critical factor in the prevention of growth depression by alfalfa meals. The failure of plant sterols to increase blood sterol concentration was interpreted as indicating that the chick behaves like the mouse in that it is unable to absorb plant sterols to any appreciable extent.

To identify further the growth-depressing action of alfalfa meal with its saponin content, the effect of saponins was determined. Two different saponin preparations, both derived from *Quillaja saponaria*, were added to the basal diet at levels ranging from 0.1 to 0.5 per cent. Both preparations produced growth depression similar to that obtained with alfalfa meal, and a combined dietary supplement of cholesterol and cottonseed

oil was again effective in restoring normal growth.

Although the harmful agent or agents in alfalfa meal have not been identified, their close similarity of behavior to that of saponins, and the observation that saponins will produce similar harmful effects strengthens the view that these are actually the factors responsible for the growth-depressing action of alfalfa meal in chick diets.

Peterson suggests that the beneficial effects of cholesterol or other sterols is due to the formation of an insoluble sterol-saponin compound in the digestive tract and that dietary fat facilitates the formation of this compound by its solubilizing action on the cholesterol in the diet.

Whether or not the effects of alfalfa meal on growth or egg production in chickens can be considered a practical problem is questionable because the harmful effects are generally absent unless the diet contains 10 per cent or more of alfalfa meal. It is commonly agreed that more than 5 to 7 per cent of alfalfa meal in the diet of chicks would be disadvantageous because of its fiber content. The recent work of Peterson indicates, however, that the increased fiber present in diets containing as much as 20 per cent of alfalfa meal did not adversely influence the growth of chicks when the diet was fortified with sterols and cottonseed oil.

THE MECHANISM OF PHOSPHORUS HOMEOSTASIS

Nutrition is concerned not only with the ingestion, absorption and utilization of nutrients, but also with their excretion. For example, adequate calcium and phosphorus nutrition requires an adequate intake of these nutrients, their absorption, and their use in body tissue metabolism. Nevertheless, unless excretion is controlled, inordinate losses of these metabolites might occur so that they would not be available for nutritional purposes. The agents necessary to control excretion and thus maintain the internal environment are in many instances endocrine in nature. Thus, the excretion of sodium and potassium is, at least in part, under adrenal cortical control. The renal excretion of phosphorus has been thought to be under the control of the parathyroid glands. However, the mechanism by which the parathyroids maintain phosphorus homeostasis has not been elucidated.

The effect of altering dietary phosphorus intake and administering parathyroid hormones upon the plasma inorganic phosphorus concentration, the urinary excretion of phosphorus, and its glomerular filtration and tubular reabsorption has been investigated, both in man and in experimental animals, by J. D. Crawford *et al.* (*J. Clin. Invest.* **29,** *1448 (1950)*). In experimental animals the effect of parathyroidectomy was also studied.

Rats were used as experimental animals and were fed on a completely synthetic diet (purified casein was the source of protein), fortified with ammonium phosphate in varying amounts. It was first observed that whereas intact animals maintained an essentially normal plasma inorganic phosphorus concentration in spite of alteration in dietary phosphorus varying from less than 0.5 g. to 8.0 g. per square meter per day, the parathyroidectomized animals had a normal plasma inorganic phosphorus concentration only when the lowest amount of phosphorus was fed. Increases of phosphorus intake in these animals up to approximately 2 g. of phosphorus per square meter per day resulted in high plasma phosphorus concentration, tetany, and death. This was accompanied by an increased glomerular filtration although there was almost complete failure to excrete phosphorus in the urine. Therefore, increased tubular reabsorption ac-

counted for the high plasma levels and this was associated with a ratio of tubular reabsorption to glomerular filtration which varied little from unity. In contrast, the ratio of the phosphorus reabsorbed to phosphorus filtered in the intact animals varied directly with the phosphorus intake. Thus at low levels of phosphorus intake there was almost no urinary excretion and marked reabsorption, whereas at high levels of phosphorus intake, reabsorption was so decreased that nearly all of that consumed was excreted.

The human subjects all received a diet containing a known content of phosphorus which could be augmented at will. The first study concerned 4 subjects with different phosphorus intakes and varying amounts of administered parathyroid extract. Again a variable effect on glomerular filtration and tubular reabsorption of phosphorus was observed. However, in all instances the administration of parathyroid extract caused within an hour, and lasting for about an hour, a striking drop in the ratio of phosphorus reabsorbed to phosphorus filtered. This again indicated that the homeostatic function of the parathyroid was not related to glomerular filtration or to tubular reabsorption of phosphorus separately, but to the ratio of the two, irrespective of the absolute value for either. In this way phosphorus excretion was adequately controlled.

Dietary phosphorus intake was abruptly increased from a low level to about 3 g. per square meter per day in further studies on 2 of the normal individuals. This was followed by an irregular rise in glomerular filtration of phosphorus, a gradual increase in urinary phosphorus excretion, and again was accompanied by a rapid fall in the ratio of phosphorus reabsorbed to phosphorus filtered. This ratio fell from almost unity to as low as 0.15.

Additional studies on 3 of the normal individuals indicated that increases in the phosphorus content from very low values up to 2 to 3 g. per square meter per day produced irregular increases in the serum inorganic phosphorus concentration which were not absolutely related to the phosphorus intake. The glomerular filtration of phosphorus likewise increased as the intake was raised, and also bore a closer relation to the intake and serum phosphorus concentration than to the urinary excretion. The latter, as before, most closely paralleled the ratio of tubular reabsorption to glomerular filtration. Thus, this ratio decreased consistently in these 3 studies with proportionate increases in phosphorus intake.

The authors conclude that in normal individuals the parathyroid glands are dynamic organs of homeostasis with respect to phosphorus metabolism, and cause adaptations to changing dietary intakes of phosphorus within a very short time. The serum phosphorus concentration, which is maintained at relatively constant levels in normal individuals, appears to be under control of the parathyroid glands and increased phosphorus in the blood acts as a stimulus to increased parathyroid activity.

PANTETHINE, PANTOTHENIC ACID AND THE *LACTOBACILLUS BULGARICUS* FACTOR

In 1949, W. L. Williams, E. Hoff-Jørgensen, and E. E. Snell (*J. Biol. Chem.* **177**, *933 (1949)*) reported the discovery of a new growth factor required by a strain of *Lactobacillus bulgaricus* for growth on a nutrient medium containing each of the previously known bacterial growth factors. This substance, which was designated as the *Lactobacillus bulgaricus* factor or LBF, was widely distributed in nature; yeast extract and refined liver extracts were among the best source materials found. By extraction with butanol, followed by successive adsorptions and elutions from charcoal and magnesium

silicate, fractions were obtained from a yeast extract that were 150 to 300 times as active as the starting material, on a weight basis, in promoting growth of *L. bulgaricus*. The active growth factor in these concentrates was readily destroyed by either acids or alkalis, was not destroyed by treatment at room temperature with nitrous acid, and appeared to be neither strongly acidic nor strongly basic. The amounts of such concentrates required for growth were relatively small, a fact that suggested the active substance was of vitamin-like character.

Subsequently, E. Kitay and E. E. Snell (*J. Bact.* **60,** *49 (1950)*) showed that this same growth factor, LBF, was required together with vitamin B_{12}, desoxyribosides, and unsaturated fatty acids, for growth of many strains and species of lactic acid bacteria that had not been grown previously in media of known composition.

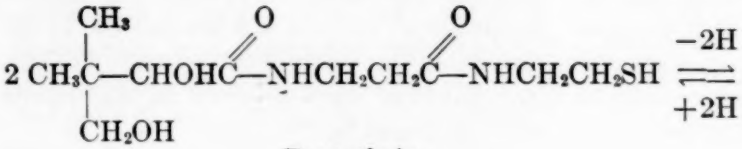
In the meantime, R. A. Rasmussen and co-workers (*Proc. Soc. Exp. Biol. Med.* **73,** *658 (1950)*) showed that several microorganisms that grew in the absence of added LBF synthesized the substance during growth and secreted it into the growth medium. The growth medium of one organism in particular—*Ashbya gossypii*—was a significantly better source material for the growth factor than any previously reported, and concentrates of LBF were subsequently prepared from this source material rather than from yeast extract. These authors showed by chromatographic experiments that LBF occurred naturally in several distinct forms. Five such forms could be detected in a yeast extract, three in the culture filtrate from *Ashbya gossypii*. Of these several naturally occurring forms of the growth factor, only one appeared to be present in concentrates prepared by a combination of procedures (not yet reported) involving adsorption on charcoal and elution with butanol. C. L. Long and W. L. Williams (*J. Bact.* **61,** *195 (1951)*) later reported even more forms of the growth factor—up to seven—in culture filtrates from certain other microorganisms.

An important clue to the nature of the growth factor was reported independently and simultaneously by R. A. McRorie, P. M. Masley, and W. L. Williams (*Arch. Biochem.* **27,** *471 (1950)*) and by G. M. Brown, J. A. Craig, and E. E. Snell (*Arch. Biochem.* **27,** *473 (1950)*). These authors found that large amounts of pantothenic acid—amounts up to 100 times higher than had previously been reported to be required for other microorganisms, and consequently much higher than the amount of this vitamin included in the test media—permitted growth of the test organisms in the absence of LBF, thus suggesting a metabolic relationship of the growth factor to pantothenic acid. That pantothenic acid was not itself the growth factor was obvious from the fact that a concentrate of LBF 25,000 times more active than yeast extract was inactive as a substitute for pantothenic acid for the yeast, *Saccharomyces carlsbergensis*, but was several hundred times more active than pantothenic acid in supporting growth of *Lactobacillus helveticus* (Brown, Craig and Snell, *loc. cit.*). The latter group also showed that by digestion with an enzyme preparation from liver, pantothenic acid could be liberated from their LBF concentrates in amounts over 50 per cent by weight of the concentrates. Such digestion, of course, resulted in loss of LBF activity for *L. helveticus*.

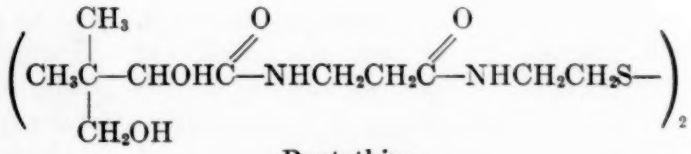
It was thus demonstrated conclusively that the various naturally occurring forms of LBF were combined forms of pantothenic acid. It has been known for some time that much of the "bound" pantothenic acid of tissues is present in a compound of unidentified structure, termed coenzyme A, a catalyst required for various acetylation reactions within the body (*Nutrition Reviews* **5,** *331 (1947)*; **9,** *78 (1951)*). Although coenzyme A itself had no LBF activity, a substance with high LBF activity was liberated from the coenzyme by digestion with intestinal phosphatase (Brown, Craig and Snell, *loc. cit.*). Such treatment had previously been shown to eliminate the coen-

zymatic activity of coenzyme A without liberating pantothenic acid in free form (*Nutrition Reviews* **7,** *112 (1949)*). Thus LBF appeared to be a fragment of the coenzyme A molecule, which, unlike coenzyme A itself, possessed high growth-promoting activity for certain bacteria that lack the ability to utilize free pantothenic acid efficiently.

In a recent preliminary publication, E. E. Snell and co-workers (*J. Am. Chem. Soc.* **72,** *5349 (1950)*) describe evidence which led them to believe that their highly purified concentrates of LBF contained pantothenic acid bound together in amide linkage with β-mercaptoethylamine. The latter product can be considered as a decarboxylation product of the amino acid cysteine, and had not been previously known to occur in nature. Synthesis of this amide showed that it did, indeed, possess high LBF activity. The synthetic product appears to exist in two forms, one of which contains a free —SH group and was named *pantetheine*, the other of which is the corresponding disulfide (—S-S—) derived from pantetheine by mild oxidation, and named *pantethine*. These products have the following formulas:



Pantetheine



Pantethine

Pantethine appeared to be identical with the active substance present in their most active LBF concentrates from natural materials.

Additional evidence for this identity is provided in a recent article by J. A. Craig and E. E. Snell (*J. Bact.* **61,** *283 (1951)*). The ability of pantethine, a concentrate of LBF prepared from natural materials, pantothenic acid, and a coenzyme A concentrate to support growth of 22 different yeasts and lactic acid bacteria in a pantothenic acid-free medium was determined. The synthetic pantethine and the LBF concentrate possessed identical activities, within experimental error, for each of these organisms. Coenzyme A was essentially inactive as a source of pantothenic acid for all but one of these organisms. Like coenzyme A, pantethine failed to support growth of any of 6 yeasts tested, for each of which pantothenic acid was highly active. For one group of lactic acid bacteria (*e.g., Lactobacillus arabinosus, Streptococcus faecalis*) pantethine, though active, was somewhat less active than pantothenic acid. For another large group of these bacteria, however, (*e.g., L. bulgaricus, L. acidophilus*, etc.) pantethine was from 50 to over 400 times as active in supporting growth as was pantothenic acid.

The discovery, purification, and synthesis of pantethine assumes considerable importance not only because of its own enhanced growth-promoting properties for many microorganisms, but also because it provides an important clue to the structure of coenzyme A itself, and of the several other forms of LBF that occur naturally. Nothing has been reported concerning the chemical nature of these additional forms. Chromatographic evidence (Long and Williams, *loc. cit.*) indicates that at least three substances with LBF activity are formed by treatment of coenzyme A with intestinal phosphatase. Whether any of these three zones corresponded to pantethine was not reported.

Similarly, the relationship of pantethine and of other forms of LBF to the pantothenic acid nutrition of higher animals remains largely to be investigated. Crude concentrates of this factor showed growth-promoting activity for chicks on rations including all of the known vitamins (Rasmussen *et al.*, *loc. cit.*); whether this activity is shown by the more highly purified concentrates or by pantethine itself, has not been reported. It is known that naturally occurring bound forms of pantothenic acid are utilized by rats (*Nutrition Reviews* **7,** *112 (1949)*). Hence it would be expected that LBF would have vitamin activity for this species. This supposition has been experimentally confirmed by R. A. McRorie, F. W. Sherwood, and W. L. Williams (*Proc. Soc. Exp. Biol. Med.* **75,** *392 (1950)*), although the relative activity of LBF and pantothenic acid cannot be ascertained from their data.

Whether LBF will prove to possess for animals any types of physiologic activity that exceed (or are not shared by) pantothenic acid itself remains for future research to determine. Certainly the investigations described above open a new chapter in our knowledge of the metabolism of pantothenic acid, and continued investigations of those problems not yet solved should yield results of considerable interest to the biochemist and nutritionist.

PROTEIN SUPPLEMENTS IN THE FEEDING OF PREMATURE INFANTS

Milk substitutes have aided greatly in providing adequate nutrition for infants and children allergic to milk. Strained meats and casein hydrolysates are two sources of protein that have been used for this purpose. These same proteins have been used in the feeding of premature infants. T. R. C. Sisson, A. F. Emmel, and L. J. Filer (*Pediatrics* **7,** *89 (1951)*) have studied nitrogen absorption and utilization by premature infants receiving milk diets supplemented with strained meat, or containing meat as the sole source of protein (*Nutrition Reviews* **8,** *208 (1950)*). The nitrogen absorption was 90.6 per cent on formulas consisting of half-skimmed milk plus 10 per cent added carbohydrate. On a similar formula in which meat supplied 30 per cent of the protein intake, the absorption of nitrogen was 86.0 per cent. When meat was the sole source of protein, the nitrogen absorption was 82.2 per cent. Nitrogen utilization, however, was greater in the infants receiving all or part of their protein in the form of strained meat. Consequently the nitrogen retention was comparable for both groups.

The absorption of fat by the infants receiving the meat-supplemented diet was not greatly different from that observed in the control group. It averaged 60.4 and 68.7 per cent respectively. Calcium and phosphorus retentions on the meat-milk diets were lower than on the milk diets alone, although the all-meat formula was supplemented with calcium and phosphorus to approximate the composition of milk. Positive iron balances were obtained in the infants receiving meat in their diet.

The meat supplements were well tolerated and no gastrointestinal disturbances or sensitivity developed in the 14 infants receiving them. Similar weight gains occurred in all of the groups.

Casein hydrolysates have also been used to increase the protein intake of premature infants (W. F. Young *et al.*, *Arch. Dis. Childhood* **25,** *31 (1950)*). An enzymatic hydrolysate of casein was used to supplement evaporated milk formulas and supplied 55 to 60 per cent of the protein intake. Gain in weight was the main criterion of protein utilization in this study. The weight gains for the first eight weeks of life of premature infants whose birth weights were over 1600 g. were

approximately the same for both the supplemented and control groups. The smaller infants receiving formulas containing the casein hydrolysate and supplying 7 g. of protein per kilogram per twenty-four hours and 128 calories per kilogram per twenty-four hours grew satisfactorily. Those receiving formulas lower in calories (90 to 126 calories per kilogram per twenty-four hours) did not grow as rapidly nor was their growth rate increased by increasing their protein intake from 3.0 to 5.0 or more g. per kilogram per twenty-four hours with casein hydrolysate. The authors suggest that this poor response may be due to the use of protein for energy production rather than for growth in the latter group. They also point out that the casein hydrolysate given to the infants receiving fewer calories had a lower mineral content than the preparation which led to satisfactory growth. Metabolic studies, especially mineral balances, would have added greatly to the value of this investigation.

M. S. Feinstein and C. A. Smith (*Pediatrics* **7,** *19* (*1951*)) measured nitrogen absorption in 5 premature infants alternately fed either calcium caseinate or an enzymatic casein hydrolysate as a supplement to an evaporated milk, Karo, and water formula. The casein hydrolysate supplied 33 to 46 per cent of the nitrogen intake provided at a level of 5 to 8 g. of protein per kilogram per twenty-four hours. Three of the 5 infants weighed less than 1600 g. at birth. The coefficient of nitrogen absorption did not differ between the two supplements. It varied from 86 to 94 per cent for both types of supplementation. This compares with the observations already discussed and confirms similar findings by S. Madey and J. Dancis (*Ibid.* **4,** *177* (*1949*)). The weight gains of these infants were satisfactory. These findings support the belief that protein digestion is not impaired in healthy premature infants.

Although the protein in strained meats and enzymatic casein hydrolysates appears to be digested and utilized by the premature infant as well as that supplied by milk, the latter product is a preferred source of calcium and phosphorus. Since the superiority of these preparations over half-skimmed milk formulas such as that studied by H. H. Gordon, S. Z. Levine, and H. McNamara (*Am. J. Dis. Child.* **73,** *442* (*1947*)) has not been demonstrated, many physicians will recommend that their use should be reserved for infants unable to take milk. For this small group of infants, however, suitable milk substitutes are badly needed, and these studies indicate considerable progress in developing such formulas.

COBALT AND RED BLOOD CORPUSCLES

From a hematologic standpoint, cobalt is an interesting and unique substance (G. E. Cartwright, *Blood* **2,** *256* (*1947*)). A deficiency results in anemia. The administration of small amounts to normal animals produces polycythemia (an excess of red blood cells), while the administration of large amounts depresses the formation of red blood cells.

The enzootic occurrence of cobalt deficiency in cattle and sheep has been reported from many parts of the world. The disease caused by a deficiency of cobalt is known in different countries by various names such as "enzootic marasmus" (Western Australia), "coast disease" (Southern Australia), "bush-sickness" (New Zealand), "pine-disease" (Scotland), "Grand Traverse disease" (Wisconsin and Michigan), and "hill-sick" (Florida). Clinically, these diseases are characterized by progressive emaciation, weakness, a rough coat, and pallor of the mucous membranes. Pathologically, the most significant finding is hemosiderosis of the liver, spleen and bone marrow. Blood studies reveal a marked anemia which is reported by some workers as being normocytic, by other

investigators as microcytic hypochromic, and by still others as macrocytic.

Interest in the role of cobalt in erythropoiesis has recently been renewed by the discovery that cobalt is contained in the vitamin B_{12} molecule (E. L. Rickes *et al.*, *Science* **108,** *134 (1948)*; *Nutrition Reviews* **9,** *155 (1951)*). Since only ruminants have been shown to require cobalt in the diet, speculation has arisen concerning the possibility that in these mammals dietary cobalt is essential for the synthesis of vitamin B_{12} by the microorganisms in the rumen and that the manifestations of cobalt deficiency are the consequence of a deficiency of vitamin B_{12}.

This theory has some experimental support in its favor. As mentioned above, anemia is a manifestation of cobalt deficiency and this anemia has been claimed by several workers to be macrocytic in type. It has been shown in cobalt-deficient animals that the oral administration of cobalt is considerably more effective than parenteral administration (S. E. Smith, D. E. Becker, J. K. Loosli, and K. C. Besson, *J. Animal Sci.* **9,** *221 (1950)*). L. S. Gall and co-workers (*Science* **109,** *468 (1949)*) have reported studies which indicate that the bacterial flora of the rumen is directly influenced by the dietary cobalt intake. J. Tosic and R. L. Mitchell (*Nature* **162,** *502 (1948)*) have shown that orally administered cobalt is selectively taken up by the microorganisms in the rumen. It is well established that large amounts of vitamin B_{12} are synthesized by intestinal bacteria. Finally, J. F. Filmer (*Australian Vet. J.* **9,** *163 (1933)*) has presented evidence that a factor is present in liver extract which is capable of preventing as well as curing cobalt deficiency in calves. He speculated that the action of liver extract was not due to its cobalt content but rather due to the presence of a factor which is produced only in the presence of cobalt.

This theory also has some experimental evidence against it. If the manifestations of cobalt deficiency in ruminants are due to a deficiency of vitamin B_{12} brought about by a failure of synthesis of vitamin B_{12} in the rumen by the bacteria, then it might be expected that a syndrome resembling pernicious anemia in human subjects might result. Neurologic manifestations have not been reported in cobalt-deficient cattle or sheep (Cartwright, *loc. cit.*). The bone marrow is stated to be hypoplastic rather than hyperplastic, and megaloblastic changes have not been observed.

D. E. Becker and S. E. Smith (*J. Nutrition* **43,** *87 (1951)*) have recently attempted to study the metabolic function of cobalt in ruminant nutrition by (1) determining the influence of cobalt deficiency upon the ability of lambs to digest various nutrients of the ration, and (2) by investigating the therapeutic value of pteroylglutamic acid, vitamin B_{12} and commercial liver extract in cobalt-deficient lambs.

For the digestibility studies, 12 western wether lambs were made deficient in cobalt by feeding a ration consisting of low-cobalt hay (mixed grasses), powdered whole milk, shelled yellow corn, and common salt. After seven months on this diet 6 of the lambs were fed 1 mg. of cobalt per day in the form of $CoCl_2.6H_2O$. The remaining 6 lambs were continued on the deficient ration without added cobalt. The digestion trials were initiated five weeks after the control group was given cobalt.

The results revealed that the cobalt-fed lambs digested more efficiently the ether-soluble and nitrogen-free extract fractions of the ration than did the cobalt-deficient lambs. However, the deficient lambs showed a significantly greater coefficient of apparent digestibility for the fibrous fraction of the ration than did the cobalt-fed lambs. The investigators attribute this effect to the depressed food intake and conclude that the cellulose-splitting microorganisms of the rumen are not seriously affected by a lack of cobalt.

A group of 15 deficient lambs was used to

determine the curative value of pteroylglutamic acid, vitamin B_{12}, and purified liver extract. At the time of the therapeutic trials all of the lambs were emaciated and anemic. Two lambs were not given any treatment and served as controls. During the trial period these animals lost an average of 8 pounds of body weight and the hemoglobin decreased from 11.1 to 8.8 grams per cent.

Three lambs were each given 10 mg. of pteroylglutamic acid by subcutaneous injection per day. The results on growth and hemoglobin were not significantly different from the untreated control group. Three animals each received 1 μg. of vitamin B_{12} and 1 mg. of cobalt by subcutaneous injection daily. These animals gained an average of 7 pounds of body weight but the hemoglobin decreased from 10.2 to 7.5 grams per cent. Purified liver extract, 15 injectable U.S.P. units, were given by subcutaneous injection daily to 3 lambs. The average weight gain was 12 pounds per animal and the hemoglobin level increased slightly from 8.2 to 9.5 grams per cent on the average. Two lambs were given the same amount of purified liver extract as above except that it was given orally rather than subcutaneously. These animals lost weight and the hemoglobin decreased from 8.3 to 7.2 grams per cent. The oral administration of 1 mg. of cobalt per day to 2 lambs resulted in an average weight gain of 17 pounds but again the hemoglobin decreased from 11.1 to 9.6 grams per cent.

The data presented indicate that purified antipernicious anemia liver extract contains some factor which is capable of alleviating cobalt deficiency. It is reasonable to assume that this activity is not due to the cobalt content of the extract, since the injection of a larger amount of cobalt than is contained in this extract was therapeutically ineffective. Furthermore, the oral administration of such an extract was ineffective, whereas the cobalt was more effective when given orally than when given parenterally.

A comparison of the rate of response of cobalt-deficient lambs to oral administration of cobalt and to subcutaneous injection of liver extract is noteworthy. Deficient lambs receiving cobalt orally continued to show a drop in mean hemoglobin level. In contrast, lambs receiving liver extract injections showed an immediate response, as indicated by all criteria. This difference suggests that the liver extract supplied more directly the deficient metabolite or metabolites than did oral cobalt.

In an addendum to the paper the authors state that data more recently obtained indicate that the amount of vitamin B_{12} injected into these lambs was suboptimal and that the injection of larger amounts has resulted in a positive response.

Thus it would seem likely from these studies that cobalt deficiency in lambs is essentially a vitamin B_{12} deficiency brought about by a failure of the bacteria in the rumen to synthesize vitamin B_{12}.

It is to be regretted that these workers did not study the anemia in greater detail. It would be interesting to know if the anemia morphologically resembles pernicious anemia and if the bone marrow shows megaloblastic erythropoiesis. It should also be pointed out that in no group of animals did the anemia disappear entirely.

VITAMIN REQUIREMENTS OF GERM-FREE CHICKS

The technics employed by J. A. Reyniers and co-workers in the rearing of germ-free animals in a sterile environment have been summarized previously in these pages (*Nutrition Reviews* **6,** *83 (1948)*; **8,** *212 (1950)*). In the last of these reports, the rearing of White Wyandotte bantam chickens through one complete life cycle into the second generation was reported. This notable accomplishment was achieved on a partially purified

diet which contained, however, 4 per cent of crude supplements in the form of liver and yeast extracts. The experiment proved that the intestinal flora was not essential to life, and opened the way to a definitive study of the effect of various microorganisms on the nutrition of the chicken. The presence of yeast and liver extracts in the diet prevented a decision as to whether or not nutritional factors in addition to the known vitamins were required for growth under such circumstances.

An extension of these interesting studies has now appeared (J. A. Reyniers *et al.*, *J. Nutrition* **41,** *31 (1950)*), having this latter objective as its principal purpose. Germ-free White Leghorn chicks were hatched from surface-sterilized eggs and maintained throughout the experimental period in the germ-free apparatus. Sterilized diets were fed both to these animals, and to control chicks hatched from eggs surface-sterilized in the same manner, and reared in a conventional incubator. The rations used were based on purified casein and gelatin as the proteins, appropriately supplemented with starch, minerals, vitamins A, D, E, and K, and the known B vitamins, with the exception of vitamin B_{12}. The vitamins were added to the diets in some excess to avoid the consequences of possible destruction during sterilization of the diets. Separate experiments, however, showed that thiamine was the only vitamin for which such precautions were necessary. Additional control groups included chicks hatched from sterilized and unsterilized eggs, and fed the nonsterilized rations. Most of the observations recorded were made at 4 weeks; a few of the experiments were continued six to eight weeks.

A detailed comparison of the various groups in the 8 different experiments run showed the following: (a) Sixteen germ-free chicks which received the purified diet supplemented with an enzymatic digest of fresh liver equivalent to 0.5 g. of liver twice weekly weighed an average of 265 g. at 4 weeks. Thirteen germ-free chicks receiving only the purified diet weighed an average of 267 g. at 4 weeks. This small supplement thus apparently supplied nothing of value not contained in the unsupplemented ration. (b) Twenty-two of these 29 germ-free chicks were directly comparable to 33 control chicks which received identical rations. The average weights of the 2 groups at 4 weeks were 245 and 246 g., respectively. Thus, over this experimental period, the intestinal flora of the control group neither contributed anything which promoted growth, nor anything that harmed growth to the host animals.

The results at 6 and 8 weeks were very similar to those at 4 weeks. However, the numbers of chicks continued were so small as to make the comparisons of doubtful significance.

Observations on hematopoiesis made at the termination of experiments showed no consistent differences between germ-free and control animals in hemoglobin, hematocrit, or total red blood cell values; the germ-free animals had a significantly lower white-cell count, however. An observation of considerable significance was that the ceca of germ-free chicks contained significant quantities of each of the vitamins determined—riboflavin, folacin, pantothenic acid, and ascorbic acid. Of these, only pantothenic acid and folacin were significantly lower than in the ceca of nonsterile animals. Thus, in these animals, either absorption of these vitamins from the ration was incomplete, or secretion of vitamins into the cecum occurred. In either case, previous evidence for the significance of the cecal flora in supplying vitamins to the host by synthesis, based as it was on the presence of the vitamins in the soluble contents of the cecum, is invalidated.

Over the period tested, the results appear fairly conclusive that no unidentified B vitamins are of critical importance for chick growth. However, the limitations of the experiments should be clearly recognized. Vitamin B_{12}, not specifically supplied with the present ration, is known to be required for early chick growth. In the present rations

sufficient amounts of this vitamin are undoubtedly supplied by the casein of the ration and from the eggs from which the chicks were hatched. It is possible that other food factors are similarly supplied. Nothing of the dietary history of the hens from which these eggs were obtained is given. It is quite possible that the latter contained stores of other unidentified nutrients sufficient to permit essentially normal growth in the absence of dietary supplies for the first few weeks of life. Thus, a full assessment by this technic of the role of the intestinal flora in supplying unidentified food factors, or the role of the latter in nutrition, will require even more highly purified rations, and experiments of sufficiently long duration to determine whether or not egg production by germ-free animals is possible on such rations and whether the eggs produced will hatch. Until such experiments are done, the possibility that the intestinal flora normally supply important ingredients to the host animal cannot be excluded. The importance of the present work in supplying the necessary background information for these more critical studies is obvious.

PHOSPHOLIPID SYNTHESIS IN PATIENTS WITH LIVER DAMAGE

It has been demonstrated repeatedly that when experimental animals are fed diets low in protein and lipotropic factors such as choline or methionine, they develop fatty livers, which in turn often lead to liver necrosis and fibrosis. Good quality protein and lipotropic factors have been used successfully by many investigators in the prevention of such liver injury.

As a result of this background of information from animal studies, many clinicians have adopted the use of high protein diets, and in some cases have also used the lipotropic factors in the management of human liver disease. It is generally felt that this resulted in a far better prognosis for such patients.

W. E. Cornatzer and D. Cayer (*Nutrition Reviews* **8,** *284 (1950)*) obtained evidence to support the view that lipotropic factors exert a beneficial effect in humans with liver cirrhosis. They demonstrated that the rate of synthesis of phospholipids in such patients could be stimulated by administering choline or methionine.

More recently these investigators have reported additional studies that included cases of infectious hepatitis (W. E. Cornatzer and D. Cayer, *South. Med. J.* **43,** *212 (1950)*). The cases used in the study were of three groups. The first group, 16 hospitalized persons who were free of organic liver disease or disturbed liver function, served as controls. The second group consisted of 10 patients with chronic hepatitis (Laennec's cirrhosis). All had clinical and laboratory evidence of marked impairment of liver function, half of them with jaundice and ascites, and nearly all with histories of alcoholism and inadequate diet. Group 3 consisted of 5 cases of infectious hepatitis.

The method used to determine the effectiveness of treatment with lipotropic factors consisted of injecting radioactive phosphorus (monobasic sodium phosphate) intramuscularly and taking blood samples at zero, twenty-four, forty-eight and seventy-two hours thereafter. The radioactivity of the plasma lipids was measured and the rate of synthesis of phospholipids was calculated. It was assumed for practical purposes that the plasma phospholipids are derived entirely from the liver and that the technic would therefore measure the capacity of the liver to synthesize phospholipids.

Although the rate of synthesis of phospholipids was somewhat lower in chronic hepatitis patients than in the normal individuals, the latter had considerable variation. Because of the extreme variation be-

tween individuals, the study was conducted so that each individual served as his own control. It was found that phospholipid synthesis in a given individual under controlled conditions remained fairly constant over periods of four to six months.

Single doses of choline or methionine (10 g.) produced a prompt rise in phospholipid synthesis in patients with chronic hepatitis. Normal individuals or patients with infectious hepatitis did not exhibit any change in phospholipid synthesis under similar treatment.

The results are indicative that lipotropic substances have a stimulatory effect on phospholipid synthesis in patients with chronic liver cirrhosis. The authors consider this as presumptive evidence that in such patients a choline deficiency exists. It was, however, not established whether the lipotropic substances had any influence on liver fat content or on liver function in general.

These results are a confirmation of results with experimental animals. Lipotropic substances are known to stimulate the synthesis of phospholipids by the liver in animals that are deficient in these substances, and C. Artom and W. E. Cornatzer (*J. Biol. Chem.* **171,** *779 (1947)*) demonstrated that this stimulatory effect is likewise absent in animals that are maintained on an adequate diet. It has been emphasized by many that a deficiency of lipotropic substances is implicated in various kinds of nutritional cirrhosis in man (*Nutrition Reviews* **8,** *13, 55 (1950)*).

That choline or methionine failed to stimulate phospholipid synthesis in patients with infectious hepatitis is perhaps to be expected and confirms the observations of many investigators that the lipotropic substances do not influence the course of this type of liver disease.

URINARY EXCRETION OF GLUCURONIC ACID FOLLOWING LARGE DOSES OF PARA-AMINOBENZOIC ACID

Para-aminobenzoic acid (PABA) is a substance of importance in nutrition. It occurs commonly as a constituent of natural foods and may be considered a member of the B group of vitamins. The vitamin has been shown to be included in the molecular structure of the pteroylglutamates. The mechanism of action of sulfonamide drugs is dependent upon competitive inhibition with PABA in metabolic systems and specifically interferes with incorporation of PABA in the vitamin, folic acid (*Nutrition Reviews* **8,** *260, 331 (1950)*).

Para-aminobenzoic acid was found to inhibit the growth of rickettsiae by an influence on the metabolism of parasitized cells. This action has been utilized clinically and it has been demonstrated that PABA is effective in the treatment of rickettsial diseases of man (*Nutrition Reviews* **4,** *87 (1946)*). Reasoning that malignant cells are characterized by disordered metabolism, perhaps similar to that of parasitized cells, postulation was made that malignant growth might be inhibited in a substrate containing PABA in relatively great concentration. In a clinical test of this hypothesis 10 patients with leukemia of varied cellular types were treated with large doses of PABA by C. J. D. Zarafonetis, G. A. Andrews, M. C. Meyers, and F. H. Bethell (*Blood* **3,** *780 (1948)*). The most striking result of this type of therapy was inhibition of granulocyte development in chronic myelogenous leukemia with a resultant decrease in total leukocyte counts. However, the over-all clinical effect was one of slight and temporary improvement. An interesting metabolic or nutritional event occurred in these patients. It was found that most of the patients excreted large quantities of a reducing substance in urine during the

period of ingestion of PABA in large dosage. PABA, itself, did not cause reduction of the sugar reagent and it was postulated that the apparent glycosuria was the result of an action of PABA interfering with mechanisms of phosphorylation in the kidney.

C. J. D. Zarafonetis and J. P. Chandler (*J. Lab. Clin. Med.* **37,** *425* (*1951*)), in investigating this phenomenon further, have found that the original conclusion was in error and that the reducing substance in the urine of patients receiving PABA is not glucose. Urine specimens of 270 patients have been examined. The patients suffered from a wide variety of diseases and ranged in age from 8 weeks to 94 years. The quantity of PABA administered daily varied from 0.8 to 48.0 g. In the urine of most of the patients a substance which reduced Benedict's solution was demonstrated during the period of PABA administration. Quantitative determination indicated that in twenty-four hours patients would excrete the reducing substance in amounts equivalent to several grams of glucose. One patient who was given 48 g. of PABA daily excreted reducing substance equivalent to 20 g. of glucose. It should be pointed out that the reaction with Benedict's solution was in every way similar to that given by glucose. The reducing material did not react as fructose in the resorcinol test.

Yeast fermentation tests were utilized in a further attempt to establish the identity of the reducing substance in these urines. It was found, however, that the presence of PABA in urine modified glucose fermentation by the test organism. When present in small concentrations, the vitamin stimulated yeast fermentation, while larger quantities suppressed this fermentation. For these reasons it was impossible to utilize the fermentation method in the attempt to establish the presence or absence of glucose in urines of patients during periods of treatment with PABA.

When the naphthoresorcinol test was applied to urines containing the reducing substance, a strongly positive reaction was obtained, indicating the presence of glucuronic acid in concentrations exceeding that in urines of normal individuals. When an osazone was made by treatment with the phenylhydrazine reagent, it was found that the osazone was not typical of glucose. Rather, the osazone produced had characteristics of solubility and of crystal structure of the osazone of glucuronic acid. This, therefore, established the reducing substance in urine of patients receiving PABA as glucuronic acid.

The urinary excretion of large quantities of glucuronic acid in these patients is not at all surprising since it has been repeatedly demonstrated that glucuronate synthesis and excretion are mechanisms utilized by the animal organism in eliminating organic substances from the body (H. G. Bray *et al.*, *Biochem. J.* **42,** *434* (*1948*)). The evidence presented indicated that this system of detoxification is important, also, in rapid elimination of aromatic monocarboxylic acids such as PABA. Other investigators have found that PABA undergoes acetylation in the body with subsequent excretion of this ester, while some of the substance is excreted in the free form (F. Lundquist, *Acta pharm. et toxicol.* **1,** *307* (*1945*)). Therefore, only a portion of the administered PABA must appear in the urine conjugated with glucuronic acid.

Zarafonetis and Chandler (*loc. cit.*) also observed the occurrence of hypoglycemia in certain patients administered large quantities of PABA. It was noted that patients who developed hypoglycemia usually had restricted their dietary intakes for several days because of anorexia, nausea and vomiting. It would be anticipated that hypoglycemia would usually be averted in the normal individual under such circumstances because of gluconeogenesis. Although the glucuronate excretion in these patients was substantial, it hardly was sufficient to divert such large quantities of carbohydrate precursors that hypoglycemia would result. The

authors postulate that PABA in some manner inhibits gluconeogenesis so that there is a decrease in the quantity of glucose synthesized within the body from protein. Whatever the mechanism, the observation is of interest. PABA may well represent a heretofore unrecognized tool for investigation of systems of carbohydrate metabolism.

SERUM IRON AND THE ADRENAL CORTEX

Certain specific alterations in iron metabolism occur in infection, both acute and chronic. Acute infection leads to hypoferremia, and when long continued it is followed by anemia. The anemia of chronic infection does not respond to iron and, in fact, the hypoferremia associated with infection cannot be corrected by the oral or intravenous administration of iron although the quantity of iron which can be administered intravenously is seriously limited by its toxicity. Studies in both animals and man have indicated that these phenomena are associated with a deviation of iron from the plasma to the liver and spleen but not to the site of infection as might have been expected. These results have been obtained from a series of investigations by M. M. Wintrobe and his colleagues (*Nutrition Reviews* **5,** *244 (1947)*).

Continuing these experiments, W. J. Kuhns, C. J. Gubler, G. E. Cartwright, and M. M. Wintrobe (*J. Clin. Invest.* **29,** *1505 (1950)*) utilized a new preparation of iron which was found to be nontoxic upon intravenous administration (saccharated oxide of iron). Some 14 patients were given massive doses of this iron preparation intravenously. In general each was given 1.0 g. of iron initially, followed by 0.1 g. daily for a week, varying between 0.8 g. and 2.2 g. for the period of study. One patient with an iron deficiency anemia manufactured 0.15 g. of hemoglobin per 100 cc. of blood each day (considered to be normal) following these large quantities of intravenous iron. Ten patients with severe chronic infection failed to manufacture significant quantities of hemoglobin for a thirty-day period following treatment in this manner. In some there was a moderate rise in serum iron concentration following therapy, that is, at the termination of the study, but in most of these patients with chronic infections no rise occurred.

Three other patients with severe fatal infections were studied. In 2, large amounts of iron were given intravenously, while in the third none was given. Analyses of the iron content of the liver and spleen at post mortem suggested that 46 and 88 per cent respectively of the iron administered to the 2 patients with severe infections was found in these two organs. Moreover, one patient with chronic draining empyema showed little or no increase in iron content of the drainage from the empyema after the administration of 0.3 g. of this iron preparation intravenously. These results indicate that even very large amounts of iron given intravenously to patients with chronic infection do not allow a bone marrow response or building of hemoglobin, but are followed by a deviation of the iron to the liver and spleen.

In a subsequent paper G. E. Cartwright and co-workers (*J. Clin. Invest.* **30,** *161 (1951)*) demonstrated a relation between adrenal cortical function and the level of plasma iron in dogs, some of which had been adrenalectomized.

The intact animals were given the following substances listed in order of increasing ability to produce hypoferremia: Normal saline (5 ml.), ACTH (25 mg.), aqueous adrenal cortical extract (30 to 60 ml.), epinephrine (2 mg.), histamine (25 mg.), turpentine (0.5 ml.), and turpentine (1 ml.). The hypoferremia produced by turpentine had been reported before by these workers,

but not the effect of a mild "stress" such as saline, or such adrenal cortical stimulating-replacing substances as ACTH, adrenal cortical extract, or epinephrine. Moreover, in 2 dogs fracture of the femur produced a marked hypoferremia reaching its peak at about twenty-four hours and returning to or toward normal by seventy-two hours. Two other forms of stress were used, namely formaldehyde and cold. The former was followed by hypoferremia but the latter appeared to have no consistent effect upon the plasma iron concentration.

The rapid development of hypoferremia observed in the intact animals following the injection of saline and ACTH was abolished following adrenalectomy, while that due to epinephrine and turpentine was reduced, but the effect was still present. However, when serum iron levels were followed for twenty-four hours after turpentine administration, one adrenalectomized animal given adrenal cortical extract developed hypoferremia of the same degree and as rapidly as intact animals. In contrast, another adrenalectomized animal receiving no extract developed only mild hypoferremia in the first eight hours, and the serum iron level then returned to its original value. This supports the thesis that the adrenal cortex is important in the development of hypoferremia. Investigation of the circulating white blood cells indicated that lymphopenia and eosinopenia occurred in almost parallel fashion with the hypoferremia following most of the "stressful" situations.

The authors believe their results indicate that the adrenal cortex is an important factor in the regulation of the plasma iron level and that it is particularly related to the production of hypoferremia following stress. They point out that it is possible to consider that cortical hormones stimulate the reticuloendothelial system to take up iron from the plasma.

In summary the results reported in these two papers support the previous findings of hypoferremia and anemia resulting from deviation of iron to the liver and spleen in patients with acute and chronic infection, and consequently their lack of response to large doses of iron administered intravenously. The hypoferremia of chronic infection appears to be mediated, at least in part, by the adrenal cortex.

THE CHEMICAL DETERMINATION OF VITAMIN B_{12}

The important role played by vitamin B_{12} and allied substances in animal nutrition, and in the control of pernicious anemia in human beings have been frequently treated in these pages. Because of the importance of the vitamin, methods for its determination are a necessity both to permit adequate control of the potency of vitamin preparations and feed supplements, and to permit an assessment of its distribution in nature. For this purpose, animal assay (*Nutrition Reviews* **7,** *210 (1949)*) or microbiologic assay are available. The latter procedure, because of its speed and sensitivity, has been most widely used. However, it suffers from several drawbacks, among which are the variable activities displayed by different naturally occurring forms of vitamin B_{12}, the fact that desoxyribosides and some reducing agents permit growth under the same conditions as vitamin B_{12}, etc. A specific chemical method for determination of the vitamin would thus be a welcome addition to the tools of the vitamin chemist.

The possibility of devising such a method has been explored with considerable success in a series of 4 papers by G. E. Boxer and J. C. Rickards of Merck and Co., Inc. (*Arch. Biochem.* **29,** *75 (1950)*; **30,** *372, 382, 392 (1951)*). Before the principles of the methods can be understood, it will be

necessary to review briefly certain of the known facts about the chemical nature of vitamin B_{12}.

Considering the complexity of the vitamin B_{12} molecule (analysis indicates a formula of $C_{61-64}H_{86-92}N_{14}O_{13}PCo$, and a molecular weight near 1400), steady and encouraging progress in elucidation of its chemical structure is being made. Among the fragments that arise on hydrolysis ribose, 5,6-dimethylbenzimidazole (N. G. Brink and K. Folkers, *J. Am. Chem. Soc.* **71,** *2951 (1949)*; **72,** *4442 (1950)*), 1-amino-2-propanol (D. E. Wolf, W. H. Jones, J. Valiant, and Folkers, *Ibid.* **72,** *2820 (1950)*), and hydrogen cyanide (Brink, F. A. Kuehl, and Folkers, *Science* **112,** *354 (1950)*) have been identified. The first two of these products are combined in a molecule termed α-ribazole (Brink and co-workers, *J. Am. Chem. Soc.* **72,** *1866 (1950)*).

Together with cobalt and phosphoric acid, these known fragments comprise between 40 and 50 per cent of the entire vitamin B_{12} molecule.

How these fragments are linked together within the molecule is not known. However, cobalt is present as a coordination complex (a type of chemical linkage similar to that which holds iron in the hemoglobin molecule, and magnesium in the chlorophyll molecule). In vitamin B_{12}, one of the groups bound to cobalt by a coordination link is the cyanide group. This linkage is very firm, separate tests having shown that vitamin B_{12} is wholly nontoxic in mice at levels of 1600 mg. per kilogram of body weight.

Other pigments that are clinically active against pernicious anemia, and that are chemically very similar to vitamin B_{12} are known to occur naturally. Two of these, reported separately as vitamin B_{12a} and vitamin B_{12b}, but both prepared by hydrogenation of vitamin B_{12} followed by exposure of the reaction product to air, appear on the basis of recent evidence (E. A. Kaczka, R. G. Denkewalter, A. Holland, and K. Folkers, *J. Am. Chem. Soc.* **73,** *335 (1951)*; D. Hendlin and M. H. Soars, *J. Biol. Chem.* **188,** *603 (1951)*) to be identical. This compound is also one of the important naturally occurring compounds with activity against pernicious anemia. Vitamin B_{12a} differs from vitamin B_{12} itself in that the cyano group of the latter compound has been replaced by a hydroxy group (Kaczka, D. E. Wolf, F. A. Kuehl, and Folkers, *Science* **112,** *354 (1950)*). This latter group of workers has shown that a series of vitamin B_{12}-like compounds, all retaining vitamin activity (at least for *Lactobacillus lactis*), can be prepared in which the cyano group of vitamin B_{12} is replaced either by a hydroxy group (vitamin B_{12a} or vitamin B_{12b}), a chloride ion, a sulfite ion, or any of several other groupings that can be firmly bonded to cobalt by a coordinate link. It is important that nomenclature be sufficiently flexible to reflect these facts, and for this reason the authors suggest the term *cobalamine* as a generic name for compounds of this type. Vitamin B_{12} itself can then be called *cyanocobalamine*; vitamin B_{12a} or $_b$ becomes *hydroxocobalamine*, etc. Hydroxocobalamine is readily changed chemically to cyanocobalamine by the addition of cyanide ions, and to other cobalamines by suitable treatment with other reagents.

With this background information, possible procedures for chemical determination of vitamin B_{12} may be considered (Boxer and Rickards, *loc. cit.*). First investigated was the possibility of determining the vitamin by acid hydrolysis to 5,6-dimethylbenzimidazole, followed by benzoylation of the latter and acid hydrolysis of the reaction product to yield 5,6-dimethyl-o-phenylenediamine. The latter is then caused to react either with acetylacetone to yield a purple-colored product that can be determined colorimetrically, or with alloxan to yield a fluorescent alloxazine, which is then determined fluorometrically. The first of these procedures could be made sensitive

to as little as 10 μg. of the benzimidazole (approximately 100 μg. of vitamin B_{12}); the second was from 100 to 1000 times more sensitive, allowing determination of amounts of vitamin B_{12} between 0.1 and 1.0 μg. Both procedures were reproducible to within ±1.5 to 2.0 per cent. However, they have apparently been applied only to pure vitamin B_{12}; their specificity when applied to natural materials or even to vitamin preparations enriched with vitamin B_{12} is not reported. Presumably, these procedures would be applicable to determination of each of the cobalamines.

An entirely different procedure based upon the specific determination of the small amounts of cyanide ion combined with the cobalt of vitamin B_{12} was also investigated. Such a procedure would, of course, not determine cobalamines other than cyanocobalamines, unless these were first transformed to the latter compound by appropriate chemical treatment. Several methods for removing the cyanide (as hydrogen cyanide) from cyanocobalamine were found. Treatment with a variety of reducing agents at 100° C. was effective; however, these tended also to liberate traces of cyanide from unidentified organic sources. Most useful was illumination of the vitamin B_{12} with visible light at room temperature, a treatment that quantitatively removes hydrogen cyanide from vitamin B_{12}. In summary the procedure involves the following steps: following thorough aeration of the sample to remove possible traces of hydrogen cyanide not bound to vitamin B_{12}, the sample is illuminated under specified conditions, and the hydrogen cyanide liberated is conducted by aeration through several reagents that trap volatile interfering organic materials, passing finally into alkali, which traps the cyanide. The latter is then treated with chloramine-T, which converts hydrogen cyanide to cyanogen chloride. Successive treatment of the latter with pyridine (a similar reaction is widely used for determination of niacin) and a color-developing reagent leads to a blue dye that is determined colorimetrically. Cyanide is readily determined with an accuracy of ±1.5 per cent in millimicrogram quantities. This permits ready determination of vitamin B_{12} in microgram quantities. Vitamin B_{12a} (hydroxocobalamine) can be determined following its conversion to vitamin B_{12} (cyanocobalamine) with cyanide, and removal of the excess cyanide by aeration in the absence of light. Good recoveries of each of these vitamins from mixtures of the two were achieved in pilot experiments. An incidental result of considerable interest was the finding of free or loosely bound cyanide in small amounts in almost all biologic materials.

This procedure was finally applied to the determination of vitamin B_{12} in multivitamin preparations and in refined liver extracts. Excellent recoveries of vitamin B_{12} from the former were obtained. With refined liver extracts, and with microbial extracts, the amounts of vitamin B_{12} found were either equal to, or less than, the values found by microbiologic assay of the same products, and recoveries of added vitamin B_{12} were excellent. Since cobalamines other than cyanocobalamine are known to be present in such materials, the low results were not unexpected. In some cases, hydroxocobalamine was also determined following its conversion to cyanocobalamine. In each case, the values thus obtained for the sum of the 2 compounds approached those obtained by microbiologic assay much more closely than the figure of cyanocobalamine alone, although in many instances they were still significantly lower than the microbiologic values. All of the samples were obtained on the open market, hence the lower chemical values may have been due either to losses of vitamin B_{12}-active substances on storage, or to the presence of substances active in the microbiologic assay but not determined by the chemical procedure. When approximately one mg. of vitamin B_{12} was injected intra-

muscularly or subcutaneously into a normal adult on a mixed diet, there was prompt and almost quantitative excretion of the dose in the urine within twenty-four hours, as determined chemically; half of the injected dose was excreted within three to five hours.

The development of these chemical procedures for vitamin B_{12} represents an important improvement in methodology. Correlation of the chemical and microbiologic methods, and their more confident application to metabolic studies of all types, should permit a rapid increase in our knowledge of the metabolic reactions of vitamin B_{12} in living organisms.

DIET NEEDS IN FLIGHT

Most individuals concerned with feeding people on the ground do not realize the problems and difficulties of feeding air crews and airplane passengers. The efficiency of air crew personnel must of course be maintained at a very high level, and it depends, in part, upon the availability and quality of food. Weight and space limitations for the storage and preparation of food constitute an important consideration. Abnormalities of digestion (especially due to the expansion of gas in the gastrointestinal tract), of absorption, and of intermediary metabolism of nutrients may occur, especially at very high altitudes. Moreover, nervous tension, vibrations and other phenomena peculiar to flying may alter appetite and digestion of food. An oxygen mask or complete pressurized suit often makes eating difficult or nearly impossible. The preparation as well as eating of food at high altitudes requires special methods of cooking, since the temperature may fall to 50 degrees below zero. The expanding use of air evacuation of personnel in this country and in Korea has brought attention to the problem of the nutrition of patients in flight, both those to be furnished with normal diets and those requiring special foods.

B. M. Ryan (*Military Surgeon* **108,** *125 (1951)*) has described the methods used by the Air Force to provide food before, during and after flight. A flight meal consists of food available for one man for one meal and may be supplied by a food packet, a precooked hot meal, a snack meal or a precooked frozen meal. A variety of equipment such as warming ovens, hot plates, etc., is available or can be installed in airplanes for keeping precooked hot meals or for preparing meals in flight.

The food packet (food packet, individual, combat, in-flight (I.S.-2)) consists of a number of meat and fruit components together with bread, dessert, beverage and an accessory packet. It is prepared in flight. The precooked hot meal is prepared on the ground and provided in an insulated container. More varied menus are available for this type of meal but it must be consumed within a few hours after it is prepared. The snack meal is either a simple breakfast, or for other times during the day provides sandwiches, cookies and similar foods. The precooked frozen meal furnishes a more complete and appetizing meal and can only be made available and served by special orders. Such items as roast turkey with dressing and gravy are available for this meal which is prepared in flight. In addition to these four flight meals, an air crew lunch is provided which furnishes chiefly energy.

The emphasis in the nutritional organization of these meals is not so much upon nutritional adequacy as upon supplying quick energy and appetizing meals for individuals during relatively short periods of time. The in-flight foods are designed to supply energy and to increase morale rather than to supply an optimum quantity of required nutrients.

NOTES

Letter to the Editor

Dear Sir:

May I comment on nitrogen balance studies in patients on the rice diet? In 1948 I undertook metabolic studies on three patients under treatment with the rice diet (H. L. de Oliveira, *Seara Médica* **4,** *165* (*1949*)). The conclusions drawn were that an equilibrium or even a positive value may be reached in the nitrogen balance after some weeks of uninterrupted use of Kempner's diet, and that a negative balance is characteristic of only the first periods (three to five weeks). Quite recently, D. M. Watkin (*J. Clin. Invest.* **29,** *851* (*1950*)) came to the same conclusions. It is apparent that the results of W. B. Schwartz and J. K. Merlis (*Nutrition Reviews* **8,** *352* (*1950*)) have little bearing on the problem of protein nutrition of patients on the rice diet because of the relative shortness of their balance studies.

Nutrition Reviews, in a recent note (*loc. cit.*), called attention to the differences that may exist between "rice diets and rice diets," admitting that the nitrogen intake need not necessarily be so low as that of the Schwartz and Merlis diet. The point I wish to stress, however, is the importance of the time factor in the balance studies of low-protein diets. One may not predict the effects of such a low-protein diet for months on the basis of metabolic studies made during the first days or weeks of the diet.

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Vitamin Losses in Thawing Frozen Meat

A. M. Pearson and co-workers (*Food Research* **16,** *85* (*1951*)) have reported that considerable losses of the B vitamins result if the "drip" which occurs during the thawing of frozen meat is discarded.

The meat used in the study was a good grade of beef processed by commercial methods. After five days of aging, the carcass was reduced to the usual cuts and the wholesale rib from each side was cut into steaks. Each steak was wrapped in cellophane, overwrapped with ordinary butcher paper, frozen and stored at 0° F. After varying periods of storage, representative samples were thawed for fourteen to fifteen hours and the "drip" collected for analysis.

The percentages of the total vitamins present in the frozen meat that appeared in the "drip" were calculated as follows: thiamine, 12.2; riboflavin, 10.3; niacin, 14.5; pyridoxine, 9.4; pantothenic acid, 33.3; and folacin, 8.1. Previous work of Pearson and J. I. Miller (*J. Animal Sci.* **9,** *13* (*1950*)) showed that the amount of "drip" increased as the length of frozen storage increased, and R. L. Hiner, L. L. Madsen, and O. G. Hankins (*Food Research* **10,** *312* (*1945*)) noted that slow freezing of meat increases the amount of "drip" on subsequent thawing.

Since meats supply 30 to 50 per cent of the intake of thiamine, niacin, and riboflavin in the average American dietary, it is clearly important to recognize the possible losses of these nutrients in the use of frozen meats. Cooking of frozen meats before thawing and the use of the cooking juices as soups or gravies are indicated as good nutritional practice.

Dental Caries From Food Grown In New England And Texas

A. E. Nizel and R. S. Harris (*New Engl. J. Med.* **244,** *361* (*1951*); *Arch. Biochem.* **26,** *155* (*1950*)) have demonstrated that an increase in the incidence and extensiveness of dental caries occurs in hamsters if fed a diet of corn and milk from New England rather than from Texas.

The diet consisted of corn (63 per cent), dried whole milk powder (30 per cent), alfalfa (6 per cent), and sodium chloride (1 per cent). Distilled water was allowed as desired. Four groups of 35 weanling hamsters each were given either New

England corn and New England milk, Texas corn and Texas milk, New England corn and Texas milk, or Texas corn and New England milk. The incidence and extent of dental caries was about the same for the three groups containing New England-grown products but was significantly and considerably less only when both of the important food products were grown in Texas.

There was a difference in the fluorine content of the milk from Texas and New England, the latter being much lower. Corn samples from the two areas were approximately equal in fluorine content. The all-Texas diet was considerably higher in fluorine than the all-New England diet, but the content of fluorine was not considered high enough by the authors to reduce the incidence of caries. Thirteen (unlisted) nutrients were analyzed in the two diets, but no significant differences were found except a 30 per cent greater content of riboflavin in the all-New England diet. The fact that the incidence of caries was equally high when either of the New England foods was included indicates that neither of the Texas foods alone had an inhibitory effect on caries. Thus, it was not possible to decide what factor in the diet was responsible for the reduced caries incidence in the all-Texas diet.

Vitamin C in Potatoes During Storage

The ascorbic acid content of potatoes may be very important in the dietary of many regions, particularly if fresh fruits and vegetables are at a minimum. In England, J. Barker (*New Phytologist* **49,** *11* (*1950*)) has sought to establish the magnitude and direction of changes in ascorbic acid content of potato tubers under various conditions and to determine whether such changes are associated with changes in sugar content. Contents of ascorbic acid tended to increase slightly during the later stages of tuber development. After harvest and during storage at 10°C. in peat moss, the content decreased steadily to about one-third of the value at harvest. This decrease was more rapid in potatoes harvested when immature than in those dug when the haulm was dead. During storage the hexose content increased initially but sucrose decreased. This decrease of sucrose was related almost linearly to the ascorbic acid decrease. Maturity affected this relationship, as well as the balance between sucrose and hexose sugars.

Effects of temperature were later studied by J. Barker and L. W. Matson (*New Phytologist* **49,** *283* (*1950*)). They noticed an occasional increase in ascorbic acid content when tubers were transferred from 10°C. storage either to below 5°C. or to 25°C. Examination at 1°, 5°, and 10°C. showed an initial slight and short-lived increase in ascorbic acid immediately after placing the potatoes at 1°, after which a decline occurred that was even more rapid than at 5° or 10°C. By decreasing the temperature to only slightly below 0°C. this initial rise could be greatly accentuated. Thus if the tubers were first stored at 10°C. for several months, the value (now low) could be doubled by storage at −0.6°C. Similar changes, but of lesser amplitude, were effected by changes from 10°C. storage to 25°C. temporary holding.

These observations were confined to a single variety of potato and may not be typical of the species. The changes in ascorbic acid content may have some very practical utility in the management of potato storage, especially just before they are placed on the market for quick consumption.